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Testis-derived Sertoli cells survive and provide localized immunoprotection for xenografts in rat brain

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Transplantation of neural tissue into the mammalian central nervous system has become an alt r-native treatment for neurodegenerative disorders such as Parkinson's disease. Logistical and ethical problems in the clinical use of human fetal neural grafts as a source of dopamine for Parkinson's dis ase patients has hastened a search for successful ways to use animal dopaminergic cells for human transplantation. The present study demonstrates that transplanted testis-derived Sertoli cells into adult rat brains survive. Furthermore, when cotransplanted with bovine adrenal chromaffin cells (xenograft), Sertoli cells produce localized immunoprotection, suppress microglial response and allow the bovin cells to survive in the rat brain without continuous systemic immunosuppressive drugs. These nov l features support Sertoli cells as a viable graft source for facilitating the use of xenotransplantation for Parkinson's disease and suggest their use as facilitators (i.e., localized immunosuppression) for cell transplantation in general.

Keywords: Sertoli cells, neural transplantation, Parkinson's disease, xenotransplantation, striatum

Neural transplantation of dopamine (DA)-secreting cells has resulted in cellular and functional recovery of Parkinson's disease (PD)[1,2] in animal models. Embryonic ventral mesencephalic (VM) tissue has been demonstrated to be the primary viable graft source for PD[2]. The major limiting factors of using embryonic VM for neural transplantation are the difficulty of obtaining sufficient amounts of viable tissue and ethical concerns. For this reason, alternative cell transplant technologies have been explored including polymer-encapsulated cells, non-neural cells that secrete DA (i.e., adrenal chromaffin cells) or cells genetically modified to secrete DA[3,4]. While some progress has been made with these alternative graft sources, it would be desirable to find an alternative technology capable of producing localized immunosuppression within the brain so that an unlimited supply of dopaminergic cells from animals can be used to treat humans (xenotransplantation).

The novel secretory features of Sertoli cells[5,6] from the testis and their availability make them a unique and potentially significant therapeutic cell type for providing enhanced immunoprotection and graft support in the brain. The present study examines the transplantability of Sertoli cells in the brain to determine whether they can provide immunoprotection to xenografts. We report that Sertoli cells are a vital source of immunosuppressive factors, thus allowing xenotransplantation of non-neural DA-secreting adrenal chromaffin cells with minimal central nervous system immune response. The localized immunosuppression provided by Sertoli cells has direct clinical applications since it avoids risk factors (i.e., infections) usually associated with systemic administration of conventional immunosuppressants such as Cyclosporine-A (CsA). As a cotransplant facilitator, they provide localized immunosuppression for xenotransplants. The use of Sertoli cell transplantation for other neurodegenerative disorders and the cells ability to provide local immunosuppression merits immediate investigations.

The Sertoli cell is a non-germ cell type that resides within the testis. It is clear that the testis can provide enhanced immunologic protection for some intratesticular grafts[5] and is, therefore, considered an immunologically "privileged" organ site. Sertoli cells secrete a wide variety of nutritive, trophic, and regulatory proteins, such as sulfated glycoprotein-1 and sulfated glycoprotein-2, androgen-binding proteins, metal carrier proteins, proteases, enzyme inhibitors, hormones, epidermal growth factors I and II, transforming growth factors α and β, and interleukins[6]. Sertoli cells have been shown to secrete the immunosuppressive factor (Fas (CD-95) ligand (FasL) that is known to down-regulate the immune response via the FasL/Fas induced apoptosis of activated lymphocytes[7]. This may, in part, provide the testis with its "immunoprivileged" status.

The remarkable secretory potential of this highly differentiated cell type suggests novel extratesticular functions for Sertoli cells such as a facilitator for cell transplantation. In addition to Fas L, it appears that Sertoli cells secrete factor(s) that inhibit the production of inerleuken-2, which in turn suppresses T-cell proliferation. Recently, Selawry and Cameron[8] demonstrated that transplanted Sertoli cells can create an immunologically "protected" site in the kidney capsule of diabetic rats that successfully supports pancreatic islet allografts. While the central nervous system (CNS) has been classically considered to be an immunologically privileged site[9], recent investigations have

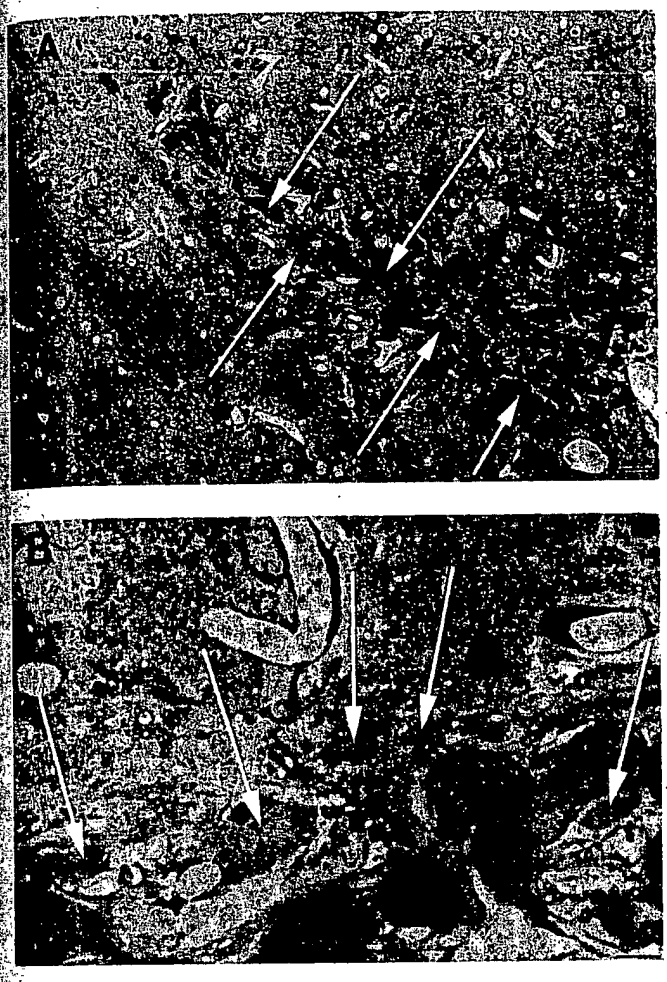

Figure 1. Light micrograph of the Sertoli cell/chromaffin cell co-graft in the striatum of the brain at 2 months posttransplantation. Light microscopy involved staining 0.5 μm with Toluidine blue. (A) Chromaffin cells (arrows) are clearly identified by their characteristic secretory granules. Calibration bar = 10 μm. (B) The boxed area in (A) at higher magnification in which Sertoli cells (arrows) are seen immediately adjacent to the electron dense chromaffin cells. Calibration bar = 5 μm.

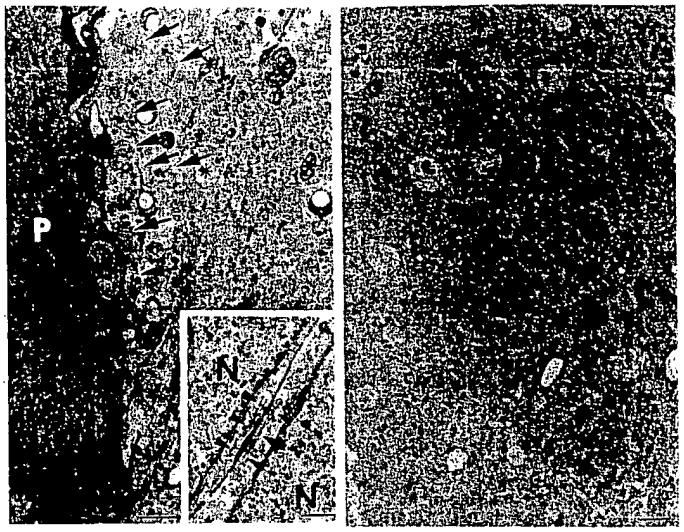

Figure 2. This light micrograph shows the striatum of a normal brain transplanted with Sertoli cells at 2 months posttransplantation. Arrows indicate Sertoli cell nuclei. Sertoli cells were identified by their characteristic nuclei and by the presence of phagocytosed latex beads. Adjacent to the implantation site of Sertoli cells (left panel), normally present microglia and astrocytes can be seen. Microglia and astrocytes are also seen in the right panel showing the contralateral control neuropril. Astrocytes are indicated with asterisks, microglia with stars. There is no evidence of edema or leukocyte infiltration adjacent to the penetration site (left panel). Calibration bar = 10 μm. Inset: This electron micrograph shows the characteristic Sertoli–Sertoli junctional complex (arrow heads) between two adjacent transplanted Sertoli cells. Transmission electron microscopy involved staining thin sections (gold-silver) with uranyl acetate/lead citrate. P, needle tract. N, Sertoli cell nucleus. Calibration bar = 0.5 μm.

demonstrated that the CNS is immunologically responsive and may significantly threaten intracerebral graft survival[10,11]. Assuming that Sertoli cells retain their immunosuppressive activity, their transplantation into other tissues may make it possible to establish immunologically privileged sites in organs other than the testis, such as the brain.

Sertoli cells are relatively easy to obtain, and thus provide sufficient amounts of viable cells for transplantation. This circumvents the problems associated with obtaining human fetal tissues and avoids the difficulty of microdissection associated with its preparation as a graft source.

Results

Sertoli cell-mediated CNS immunosuppression in xenotransplants. This experiment was designed to investigate whether Sertoli cells retain their immunoprotective activity following transplantation into the brain and subsequently provide localized immunoprotection for xenotransplantation. Intrastriatal cotransplantation of bovine adrenal chromaffin cells (xenografts) and rat Sertoli cells to one side of the brain, and bovine adrenal chromaffin cells alone to the other side of the brain were performed in adult normal rats (n=6). At 2 months posttransplantation, all animals were sacrificed and brains were examined histologically. Examination of coronal brain sections showed Sertoli cells closely associated with viable chromaffin cells (Fig. 1). There was no evidence that either Sertoli cells or chromaffin cells migrated from the original transplantation site. No surviving chromaffin cells were seen when transplanted alone.

The survival of Sertoli cells posttransplantation was initially verified by their characteristic nuclei (Fig. 2). The presence of Sertoli–Sertoli junctional complex (Fig. 2 inset), which is unique to Sertoli cells and no other type of cell–cell contact, also indicated surviving transplanted Sertoli cells. Astrocytes and microglia appeared to be within normal limits following the Sertoli cell only transplants (Fig. 2). In order to provide further evidence for survival of Sertoli cells in the brain an additional study involved normal rats (n=4), where 1 μm latex beads were added to the incubation medium of isolated Sertoli cells 24 h prior to transplantation to allow phagocytosis of the beads. These beads served as an effective marker for the grafted Sertoli cells, further verifying the presence of Sertoli cells in the brain at 1 month posttransplantation (Fig. 3). Additionally, we used DiI labeling to tag transplanted Sertoli cells in another set of normal rats (n=4). Using fluorescent microscopy to excite DiI-labeled Sertoli cells, we noted viable Sertoli cells at 1 month posttransplantation (Fig. 4). These markers clearly demonstrated viable Sertoli cells transplanted in the rat brain.

The presence of Sertoli cells resulted in a marked reduction of activated microglia at the transplantation site when compared to the contralateral transplantation site receiving only bovine adrenal chromaffin cells (Fig. 5). Analysis of the area of microglial activation surrounding each implantation site was conducted, independently by two observers using color segmentation with

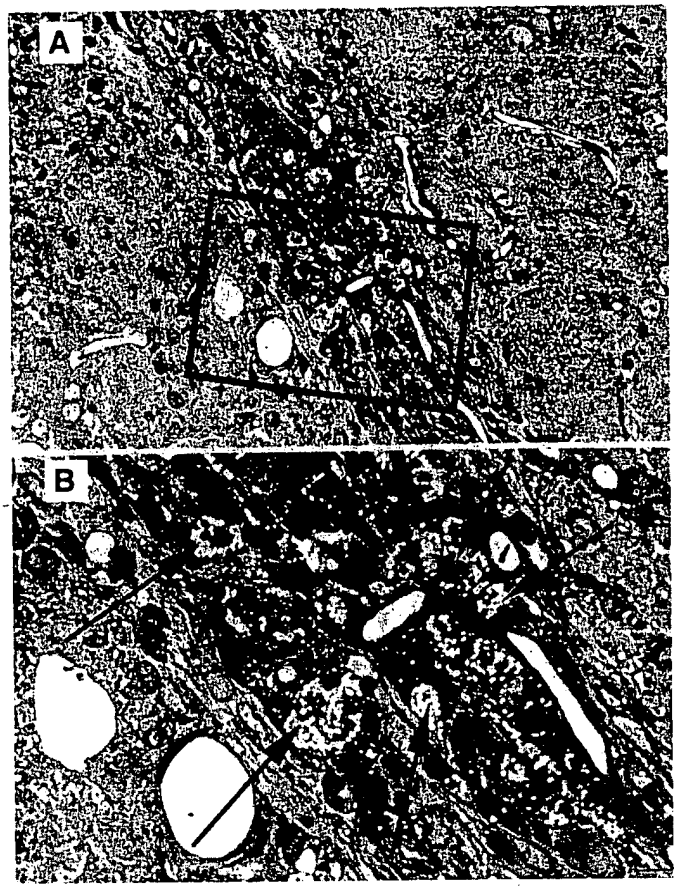

Figure 3. (A) The striatum of the brain in this light micrograph shows the needle tract (arrows) and the site of Sertoli cell transplantation. Calibration bar = 10 μm. (B) The boxed area in (A) at higher magnification in which Sertoli cells (arrows) are easily identified because of the presence of numerous 1 μm latex beads in the cytoplasm of the transplanted cells. Beads were loaded into the cells prior to transplantation. Calibration bar = 5 μm.

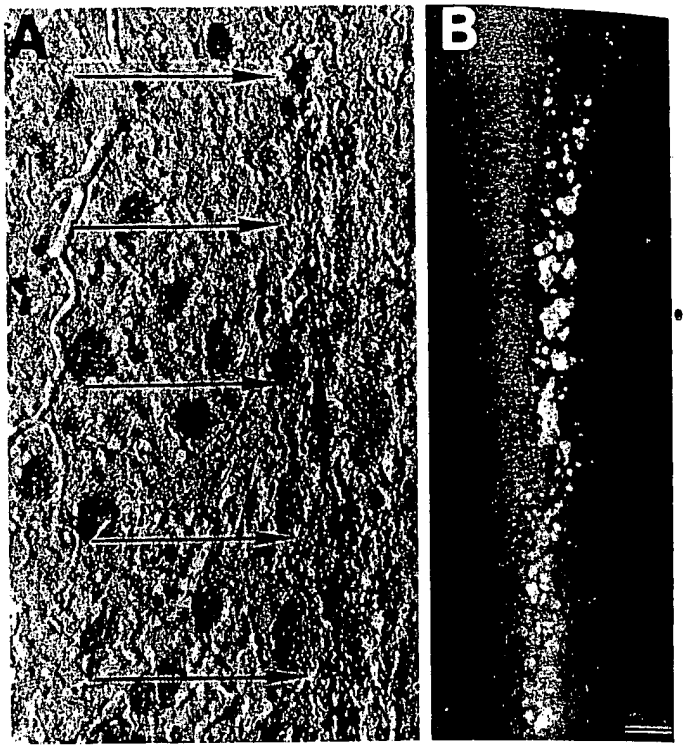

Figure 4. Photo micrographs of grafted Sertoli cells in situ labeled with DiI prior to their transplantation into the striatum of the brain. Tissue was collected at 1 month posttransplantation. (A) The needle tract (arrows) viewed with interference contrast optics and in (B) the needle tract (arrows) is imaged with UV microscopy to excite the DiI-labeled Sertoli cells. Calibration bar = 20 μm.

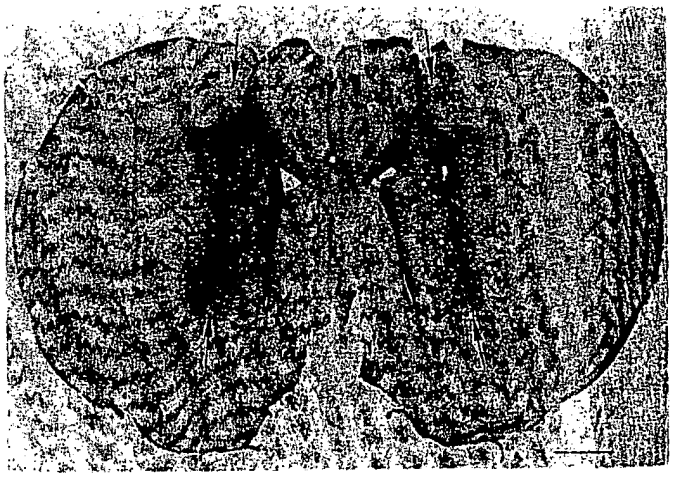

Figure 5. Lectin histochemistry showed a decrease in microglia around the transplantation sites in the striatum of brains transplanted with Sertoli cells and bovine adrenal chromaffin cells (right) as compared to the striatum of brains transplanted with bovine adrenal chromaffin cells alone (left) (2 months posttransplantation). Calibration bar = 1 μm.

computer-based image analysis system, using size and color intensity and saturation limits that are also stained by this technique. The total volume of the microglial response in the chromaffin cells alone transplanted side was 0.73±0.21 (mean±standard error of the mean) mm^3 while the microglial response on the chromaffin cells with Sertoli cells transplanted side was 0.1±0.037 mm^3 ($p<0.01$). Thus, there was a sevenfold decrease in the microglial response on the side of the brain transplanted with chromaffin cells and Sertoli cells. The suppression of this response on this side of the brain suggests that the transplanted Sertoli cells retained their immunosuppressive activity resulting in the subsequent inhibition of the CNS immune response.

Discussion

Sertoli cells grafted into the striatum of the rat brain suppressed microglial activation, and promoted survival of bovine chromaffin cell xenografts. These positive effects of Sertoli cells may be accomplished by the local secretion of nontoxic immunosuppressive factors, such as FasL, gliosis inhibiting factors and/or neurotrophic factors. A variety of cell types have been grafted into the nervous system. For example, bovine chromaffin cells have been used to alleviate cancer pain in rodent models[12]. However, xenografts in the nervous system show variable survival and require systemic CsA immunosuppression[13]. Even with long-term CsA treatment (42 days) survival of the CNS xenograft remains variable following termination of the exogenous systemic immunosuppression[1,14]. In addition to placing the patient at medical risk from systemic immunosuppression, CsA may itself be counterproductive to successful intracerebral graft acceptance. In fact, CsA may be cytotoxic to the graft[15,16]. A num-

ber of strategies have been developed to circumvent the negative side effects of systemic immunosuppression in PD[2,3,17,18]. With the possible exception of autografts, these transplantation protocols require sophisticated methods for preparation of the cells (e.g., genetic modification, polymer encapsulation). The transplantation of Sertoli cells as described in this report is a uniquely efficient and effective transplantation protocol, and appears to provide an endogenous source of local immunosuppression that does not appear to be cytotoxic to the nervous tissue.

As has been demonstrated in the diabetic model[8], transplanted Sertoli cells may survive for a long period of time in the brain and, therefore, maintain graft viability and long-term recovery.

Porcine Sertoli cells may be the appropriate source for conducting clinical trials of transplantation of Sertoli cells. The relative similarity in size between porcine Sertoli cells and human brain cells, and the high yield of Sertoli cells that can be harvested from porcine testis may prove advantageous, as well as the availability of pathogen-free pigs. Thus, the ready availability of Sertoli cells can significantly reduce the logistical and procedural problems inherent in traditional cell transplantation protocols and provide for a novel approach to cell transplantation in the brain.

Experimental protocol

Preparation of Sertoli cell and adrenal chromaffin cell suspension. Rat Sertoli cells were isolated from 16–19-day old Sprague-Dawley rats following the procedure described by Selawry and Cameron[6]. Sertoli cells for transplantation were obtained from 3-day old Sertoli cell cultures. The techniques of suspension preparation and transplantation of Sertoli cells or cotransplantation of Sertoli cells and bovine adrenal chromaffin cells were based on the procedures for striatal cell suspension preparation and transplantation described[19]. The bovine adrenal chromaffin cells were already in cell suspension when obtained from the University of Illinois (Chicago, IL). Specific details of the preparation of bovine adrenal chromaffin cell suspension have been reported[20]. All procedures were carried out under aseptic conditions. For the cotransplantation of Sertoli cells and bovine adrenal chromaffin cells, cell suspensions of Sertoli cells[6] and bovine adrenal chromaffin cells[20] were combined in a 50-ml test tube and centrifuged at 150 x G for 10 min at room temperature. The medium was decanted from the bovine adrenal chromaffin cell–Sertoli cell pellet, and fresh DMEM medium with 10% FCS was added. The cells were resuspended into a culture flask and placed in a 5% CO_2, 95% air incubator at 37°C temperature until the day of transplantation 24 h later.

Intrastriatal grafting. During the day of transplantation, the cells were resuspended into a volume of 1 ml DMEM and then drawn into a 10 µl Hamilton microsyringe that was mounted to a Kopf stereotaxic frame. The striatal transplantation coordinates were AP: 1.5 mm, ML: ±2.6 mm, DV: 6.2 mm (based on frontal atlas sections[21]). Each animal was first anesthetized with sodium pentobarbital (60 mg/kg, i.p., Sigma Chemical Co., St. Louis, MO) and placed in the stereotaxic frame. For transplantation of Sertoli cells alone or bovine adrenal chromaffin cells alone, the same procedures were followed except that the two types of cells were not combined. Each animal received 3 µl solution of bovine adrenal chromaffin cells to one side of the brain and another 3 µl solution of bovine adrenal chromaffin cells with Sertoli cells to the other side. Trypan blue exclusion was conducted before and after transplantation. Cell viability counts showed greater than 95 percent cell survival in both time periods. A mean total of greater than 5500 surviving cells was seen in the 3 µl solution of either bovine adrenal chromaffin cells with Sertoli cells, Sertoli cells alone, or bovine adrenal chromaffin cells alone.

Histological procedures. For histological examinations, animals receiving cotransplants were sacrificed at 4 or 8 weeks posttransplantation. Each animal was injected with a lethal dose of sodium pentobarbital (70 mg/kg, i.p., Sigma) and perfused intracardially with saline followed by 4% paraformaldehyde in 0.1m phosphate buffer (pH 7.4). Brains were then dissected post-fixed in 4% paraformaldehyde for 24 h and then placed in 20% sucrose in PBS overnight. The following day, the brains were sectioned (40 µm) using a Vibratome microslicer (752M Vibroslice, Campden Instruments). To visualize microglia, brain sections were reacted with *Griffonia simplicifolia* (Sigma) conjugated with horseradish peroxidase (HRP) in accord with the procedure described by Streit[22]. Twelve sections through the penetration were taken at 80 µm intervals and ditigally captured in true color for analysis. Each image was analyzed using a computer-based image analysis system (Image-Pro Plus, Media Cybernetics). Two observers independently analyzed the amount of HRP reaction product by color segmentation on each image on two separate occasions. For high resolution microscopy brain tissue containing the lesion, were post-fixed with 5% gluteraldehyde, routinely preserved for resin embedding, and thick and thin sectioned for light and electronmicroscopic analysis, respectively.

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